

**What is Claimed:**

1. A collection of antigenic polypeptides, comprising:  
at least three antigenic polypeptides that comprise 5 to 8 unique residues and include at least 4 residues, designated critical residues, selected from E, P, Q, N, F, H, T, K, L, D, wherein:  
critical residues occupy the N and C terminal positions in each polypeptide; and no more than three polypeptides in the collection contain the same four critical residues.
2. The collection of claim 1, wherein at least two polypeptides in the collection comprise the same four critical residues but each of the two polypeptides has non-critical residues at different positions; and wherein the polypeptides comprise at least 6 unique residues and at least 2 non-critical residues that are adjacent to each other.
3. The collection of claim 2, wherein the non-critical residues are selected from among Y, S and G.
4. The collection of claim 1, wherein the polypeptides comprise 6 unique residues.
5. The collection of claim 1, wherein the polypeptides comprise 7 unique residues.
6. The collection of claim 1 comprising at least 4, 5, 6, 7, 8, 9, 10, 20, 25, 30, 40, 50, 60, 70, 80, 90 or 100 members.
7. The collection of claim 1 comprising at least 200, 300, 400, 500, 750, 1000 members.
8. The collection of claim 1 comprising at least 5000 or 10,000 members.
9. The collection of claim 1, wherein the polypeptides are at least 6, 7, 8, 9, 10, 11, 12, 15, 20, 25, 30, 35, 40, or 45 amino acids in length.
10. The collection of claim 1, wherein the polypeptides are between 6 and 8 or 6 and 10 or 6 and 12 or 6 and 15 or 6 and 20 amino acids in length.

11. The collection of claim 1, wherein the polypeptides are antigenic in a non-human subject.

12. The collection of claim 11, wherein the polypeptides exhibit higher antigenicity in a non-human subject than in a human subject.

13. The collection of claim 12, wherein the non-human subject is a rodent or a bird.

14. The collection of claim 1 that is addressable.

15. The collection of claim 1, wherein the polypeptides of the collection are positionally addressable.

16. The collection of claim 1, wherein the polypeptides of the collection are immobilized on a solid support.

17. The collection of claim 1, wherein the polypeptides are conjugated to members of a library.

18. The collection of claim 17, wherein the library is selected from a nucleic acid library, a polypeptide library, a natural products library, and a combinatorial chemistry library.

19. The collection of claim 1, wherein the polypeptides of the collection are conjugated to molecules selected from the group consisting of polypeptides, nucleic acids and small organic molecules.

20. A collection of capture agent - binding partner polypeptide pairs comprising:

a collection of binding partners comprising a collection of antigenic polypeptides according to claim 1; and

a collection of capture agents, wherein the capture agents each bind to a unique binding partner within the collection of antigenic polypeptides.

21. The collection of claim 20, wherein the capture agents comprise antibodies or antibody fragments.

22. The collection of claim 20, wherein the collection of binding partners include 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 50, 100 or more polypeptides of any of SEQ ID Nos. 1-911.

23. A collection of binding partner polypeptides, comprising 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 50, 100 or more polypeptides of any of SEQ ID Nos. 1-911.

24. A fusion protein, comprising a first polypeptide or one or more amino acids conjugated to any of the polypeptides set forth as SEQ ID Nos. 1-911.

25. A fusion protein of claim 24 that is at least 6, 7, 8, 9, 10, 11, 12, 15, 20, 25, 30, 35, 40, 45 amino acids in length.

26. A method of generating highly antigenic highly specific binding polypeptides, comprising:

a) ranking amino acids based upon pre-determined criteria for antigenicity, wherein n amino acids are ranked;

b) based upon the ranking using the top m to n-1 ranked amino acids, generating all combinations of the amino acids in a polypeptide of pre-selected length m residues to produce a set S1 of polypeptides of length m residues, wherein:

n is the number of amino acids in a preselected set of possible amino acids; and

m is a length of amino acids preselected to have a minimum length to result in sufficient affinity to bind to a selected capture agent up to a length that retains specific binding to a selected capture agent; and

c) based upon pre-determined criteria for dissimilarity, selecting a subset of set of dissimilar polypeptides from set S1.

27. The method of claim 26, wherein the pre-determined criteria for antigenicity is based upon frequency of the amino acids in a pre-selected set of antigenic polypeptides.

28. The method of claim 26, wherein n is equal to 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 or 19.

29. The method of claim 26, wherein m is equal to 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30.

30. The method of claim 26, wherein  $m$  is an integer between and including 4 and 6 or 4 and 7 or 4 and 8 or 4 and 12 or 5 and 7 or 5 and 8 or 5 and 12 or 6 and 8 or 6 and 10 or 6 and 12 or 8 and 12.

31. The method of claim 26, wherein the amino acids are selected from among E, P, Q, N, F, H, T, K, L, D, S, G and Y.

32. The method of claim 26, wherein the amino acids are naturally-occurring amino acids.

33. The method of claim 26, wherein the amino acids include non-naturally occurring amino acids.

34. The method of claim 33, wherein the amino acids include non-naturally occurring and naturally-occurring amino acids.

35. The method of claim 34, wherein  $n$  is between 20 and 10,000.

36. The method of claim 26, further comprising generating a subset of polypeptides of length  $q$  residues, wherein  $q = m + r$ ;  $r$  is the number of non-critical amino acids;  $r$  is an integer equal to or greater than 1; and  $q$  is an integer greater than 4.

37. The method of claim 36, wherein the N and C terminal amino acids of the polypeptides of length  $q$  residues are critical amino acids.

38. The method of claim 37, wherein at least 2 of the non-critical amino acids are adjacent.

39. The method of claim 36, wherein  $r$  is 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10.

40. The method of claim 39, wherein  $r$  is 2, 3 or 4.

41. The method of claim 36, wherein  $q$  is an integer between 5 and 100 or 5 and 50 or 5 and 30 or 5 and 20 or 5 and 10.

42. The method of claim 26, wherein:

dissimilarity is assessed by comparing each polypeptide in the set S1 to an arbitrarily selected reference polypeptide from the set S1 by comparing corresponding critical residues based upon position in the polypeptides; and wherein polypeptides from set S1 are selected that

contain corresponding critical residues most dissimilar from the reference polypeptide; and dissimilarity refers to functional and structural dissimilarity based upon predetermined criteria.

43. The method of claim 42, wherein dissimilarity is determined by calculating a similarity score from a similarity matrix by comparing values for the corresponding critical residues in the reference polypeptide to the corresponding critical residues in polypeptides of set S1; combining the scores for the residues in each polypeptide to generate a score for each polypeptide; and selecting those below a predetermined score.

44. A collection of binding partner polypeptides, comprising 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 50, 100 or more polypeptides generated by the method of claim 26.

45. A collection of capture agent - binding partner polypeptide pairs, comprising the collection of binding partner polypeptides of claim 44 and a collection of capture agents, wherein the capture agents each bind to a binding partner polypeptide within the collection of binding partner polypeptides.

46. A kit, comprising:  
a collection of claim 44; and optionally including instructions preparing capture agents that specifically bind to members of the collection.

47. A method for synthesizing an addressable collection of polypeptides comprising:

a) providing a collection of b tags and a collection of b addressable capture agents, wherein each capture agent binds a unique tag and b is the number of tag-capture agent pairs;

b) presenting the tags in an addressable format suitable for peptide synthesis;

c) synthesizing a collection of polypeptides on the collection of tags, wherein each tag is conjugated directly or indirectly via a linker to a synthesized polypeptide, wherein each synthesized polypeptide

comprises a number of variable amino acid positions  $v$  and optionally a number  $n$  of fixed amino acid positions each designated  $N$ ;

each  $N$  can be the same or different; and

the method of synthesis comprises:

i) synthesizing a subset of  $v$  positions in a first round of synthesis to generate a collection of tag- $v_1$  polypeptides, whereby each unique tag is directly or indirectly linked to an amino acid, wherein each tag has a unique combination of amino acids at the synthesized variable positions;

ii) mixing the the collection of synthesized tag- $v_1$  polypeptides and splitting the collection of tag- $v_1$  polypeptides into  $b$  addressable first-round subsets, whereby each first-round subset contains a collection of tag- $v_1$  polypeptides representing on average every possible combination of amino acids at the synthesized variable positions;

iii) synthesizing a further subset of variable positions  $v_2$  in a further round of synthesis, such that each tag- $v_1$  polypeptide is conjugated to a unique combination of amino acids at  $v_2$  positions to generate  $b$  subsets of tag- $v_1v_2$  polypeptides;

iv) contacting the resulting subsets of tag- $v_1v_2$  polypeptides with the addressable collection of  $b$  capture agents to produce an addressable collection of synthesized polypeptides.

48. The method of claim 47, wherein  $v$  is equal to 4 and the subset of variable positions synthesized in the first round is equal to 2.

49. The method of claim 47, further comprising:

d) incubating the addressable collection of synthesized polypeptides or a subset thereof with one or more collections of molecules under conditions where one or more molecules specifically binds to the synthesized polypeptides.

50. The method of claim 49, wherein the collections of molecules comprise molecules selected from the group consisting of antibodies, fragments of antibodies and polypeptides.

51. The method of claim 47, wherein the collection of b capture agents are conjugated to a solid support.

52. The method of claim 51, wherein b collections of b capture agents are conjugated to a solid support.

53. The method of claim 47, wherein the capture agents comprise antibodies or antibody fragments.

54. The method of claim 47, wherein the synthesized polypeptides are of length d, wherein  $d = n + v$  and n is an integer equal to or greater than 1.

55. The method of claim 54, wherein n is 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12.

56. The method of claim 47, wherein v is 4, 5, 6, 7 or 8.

57. The method of claim 47, wherein the synthesized polypeptides are highly antigenic highly specific polypeptides.

58. A method for synthesizing an addressable collection of molecules comprising:

a) providing a collection of b tags and a collection of b addressable capture agents, wherein each capture agent binds a unique tag and b is the number of tag-capture agent pairs;

b) presenting the tags in an addressable format suitable for chemical synthesis;

c) synthesizing a collection of molecules, wherein the molecules are synthesized on starting molecules;

each tag is conjugated directly or indirectly via a linker to a starting molecule;

each synthesized molecule comprises a number of variable constituent positions X conjugated to the starting molecule;

and the method of synthesis comprises:

i) synthesizing a subset of X positions  $X_1$  in a first round of synthesis to generate a collection of tag- $X_1$  molecules, whereby

each unique tag is conjugated to a unique combination of constituents at the synthesized  $X_1$  positions;

ii) mixing the collection of synthesized tag- $X_1$  molecules and splitting the collection of tag- $X_1$  molecules into  $b$  addressable first-round subsets, whereby each first-round subset contains a collection of tag- $X_1$  molecules representing on average every possible combination of constituents at the synthesized  $X_1$  positions;

iii) synthesizing a further subset of constituent positions  $X_2$  in a further round of synthesis, such that each first-round subset is conjugated to a unique combination of constituents at  $X_2$  positions to generate  $b$  second-round subsets;

iv) contacting the resulting tag- $X_1X_2$  with an addressable collection of  $b$  capture agents to produce an addressable collection of synthesized molecules.

59. The method of claim 58, wherein the synthesized molecules are selected from the group consisting of nucleic acid molecules, polymers, biopolymers, polypeptides, and small organic molecules.

60. The method of claim 58, wherein the starting molecule is a pharmacophore.

61. The method of claim 58, wherein the starting molecule is a monomer and the synthesized molecules are polymers.

62. The method of claim 58, wherein the tags are highly antigenic highly specific polypeptides.

63. The method of claim 58, wherein the capture agents comprise antibodies or antibody fragments.

64. The method of claim 58, wherein the tags comprise any of the polypeptides set forth in SEQ ID Nos. 1-911.